if it is greater than 5,500 theoretical plates.

(C) *Resolution.* The resolution (*R*) between the peak for dexamethasone and its nearest eluting impurity is satisfactory if it is not less than 1.1.

(D) *Coefficient of variation.* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph(b)(2)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the dexamethasone content of the container as follows:

$$\text{Milligrams of dexamethasone per container} = \frac{A_u \times P_a \times d}{A_s \times 1{,}000}$$

where:

A_u=Area of the dexamethasone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the dexamethasone peak in the chromatogram of the dexamethasone working standard;

P_s=Dexamethasone content in the dexamethasone working standard solution in micrograms per milliliter; and

d=Dilution factor of the sample.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

(5) *Tobramycin identity.* Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows; decant 1 milliliter into a test tube. Add 100 milligrams of sodium sulfate to the test tube and shake until the sodium sulfate has been dispersed. Centrifuge to obtain a clear supernatant. Use the supernatant as the sample solution.

(6) *Dexamethasone identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the dexamethasone working standard.

[54 FR 13879, Apr. 6, 1989, as amended at 59 FR 8399, Feb. 22, 1994]

§ 444.380d Tobramycin-dexamethasone ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin-dexamethasone ophthalmic ointment contains in each gram, tobramycin equivalent to 3.0 milligrams of tobramycin and 1.0 milligram of dexamethasone, with a suitable preservative in a suitable and harmless white petrolatum base. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its dexamethasone content is satisfactory if it is not less than 90 percent and not more than 110 percent of the number of milligrams of dexamethasone that it is represented to contain. It is sterile. Its moisture content is not more than 1.0 percent. It passes the test for metal particles. It passes the identity tests for tobramycin and dexamethasone. The tobramycin used conforms to the standards prescribed by § 444.80(a)(1), except heavy metals. The dexamethasone used conforms to the standards prescribed by the U.S. Pharmacopeia XXII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The dexamethasone used in making the batch for all U.S. Pharmacopeia XXII specifications.

(C) The batch for tobramycin potency, dexamethasone content, sterility, moisture, metal particles, tobramycin identity, and dexamethasone identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Tobramycin potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of distilled water and shake well. Allow the layers to separate. Remove the distilled water layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of distilled water. Combine the extractives in a suitable volumetric flask and dilute to volume with distilled water. Further dilute an aliquot with distilled water to the reference concentration of 2.5 micrograms of tobramycin per milliliter (estimated).

(2) *Dexamethasone content.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 254 nanometers, a column packed with microparticulate (3 to 10 micrometers in diameter) reversed phase packing material such as octadecyl hydrocarbon bonded silicas, a flow rate of 1.5 milliliters per minute, and an injection volume of 20 microliters. Mobile phase, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Mobile phase.* Mix acetonitrile:water (45:55) and adjust if necessary by reducing the amount of acetonitrile to increase retention, or by increasing the amount of acetonitrile to decrease the retention of the solute. Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard and sample solutions and resolution test solution*—(A) *Working standard solution.* Accurately weigh approximately 20 milligrams of the dexamethasone working standard into a 100-milliliter volumetric flask containing methonol:water (75:25). Shake until dissolved. Dilute to volume with methanol:water (75:25). Transfer 10.0 milliliters of this solution to a separatory funnel containing approximately 50 milliliters of hexane. Shake until homogeneous. Add 15.0 milliliters of methanol:water (75:25) and shake well. Allow the layers to separate. Remove the lower (methanol:water) layer and repeat the extraction twice more with 15.0 milliliters of methanol:water (75:25). Collect the extractives in a 50-milliliter volumetric flask. Dilute to volume with methanol:water (75:25) to obtain a solution of known concentration containing approximately 40 micrograms of dexamethasone per milliliter.

(B) *Sample solution.* Accurately weigh approximately 2.0 grams of the sample and place into a separatory funnel containing approximately 50 milliliters of hexane. Shake until homogeneous. Add 15.0 milliliters of methanol:water (75:25) and shake well. Allow the layers to separate. Remove the lower (methanol:water) layer and repeat the extraction twice more with 15.0 milliliters of methanol:water (75:25). Collect the extractives in a 50-milliliter volumetric flask. Dilute to volume with methanol:water (75:25) to obtain a solution of known concentration containing approximately 40 micrograms of dexamethasone per milliliter (estimated).

(iii) *System suitability requirements*—(A) *Asymmetry.* The asymmetry (A_s) is satisfactory if it is not more than 1.6 at 10 percent of peak height.

(B) *Efficiency of the column.* The efficiency of the column (n) is satisfactory if it is greater than 5,500 theoretical plates.

(C) *Resolution.* The resolution (R_s) between the peak for dexamethasone and its nearest eluting impurity is satisfactory if it is not less than 1.1.

(D) *Coefficient of variation.* The coefficient of variation (*RSD* in percent) of 5 replicate injections is satisfactory if it

is not more than 2.0 percent. If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter.

(iv) *Calculations.* Calculate the dexamethasone content as follows:

$$\text{Milligrams of dexamethasone per gram} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the dexamethasone peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the dexamethasone peak in the chromatogram of the dexamethasone working standard;

P_s=Dexamethasone content in the dexamethasone working standard solution in micrograms per milliliter;

d=Dilution factor of the sample; and

n=Number of grams of sample assayed.

(3) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in § 436.20(e)(1).

(4) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(5) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

(6) *Tobramycin identity.* Proceed as directed in § 436.318 of this chapter, except prepare the sample for assay as follows: Weigh approximately 1 gram of the sample into a test tube. Add 1 to 2 milliliters of chloroform to the test tube and shake vigorously to dissolve the ointment. Centrifuge for approximately 15 minutes to clearly separate the layers. Use the top (aqueous) layer in the procedure.

NOTE: If an oily film remains on the top of the aqueous layer and interferes with sampling, the aqueous layer may be transferred to another test tube and washed with an additional 1 to 2 milliliters of chloroform.

(7) *Dexamethasone identity.* The high-performance liquid chromatogram of the sample determined as directed in paragraph (b)(2) of this section, compares qualitatively to that of the dexamethasone working standard.

[55 FR 617, Jan. 8, 1990]

§ 444.380e Tobramycin-fluorometholone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tobramycin-fluorometholone acetate ophthalmic suspension is an aqueous suspension containing, in each milliliter, 3.0 milligrams of tobramycin and 1.0 milligram of fluorometholone acetate in a suitable and harmless aqueous vehicle. It contains one or more suitable and harmless dispersants, preservatives, buffers, and tonicity agents. Its tobramycin potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of tobramycin that it is represented to contain. Its fluorometholone acetate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of fluorometholone acetate than it is represented to contain. It is sterile. Its pH is not less than 6.0 and not more than 7.0. It passes the identity tests for tobramycin and fluorometholone acetate. The tobramycin used conforms to the standards prescribed by § 440.80(a)(1) of this chapter, except heavy metals. The fluorometholone acetate used conforms to the standards prescribed in the U.S. Pharmacopeia XXII.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The tobramycin used in making the batch for potency, moisture, pH, identity, and residue on ignition.

(B) The fluorometholone acetate used in making the batch for all requirements in U.S. Pharmacopeia XXII.

(C) The batch for tobramycin potency, fluorometholone acetate content, sterility, pH, tobramycin identity, and fluorometholone acetate identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(A) The tobramycin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(B) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.